

5
METHODS AND COMPOSITIONS
FOR THE TREATMENT OF PANCREATITIS

10 Field of the Invention

66040-333350

The present invention includes methods and compositions for the treatment of acute pancreatitis. In a preferred embodiment the invention concerns the use of agents to
15 reduce or prevent the secretion of pancreatic digestive enzymes within the pancreas. Such agents are targeted to pancreatic cells, and serve to prevent the exocytotic fusion of vesicles containing these enzymes with the plasma
20 membrane. The invention is also concerned with methods of treating a mammal suffering from pancreatitis through the administration of such agents.

Background of the Invention

25 Pancreatitis is a serious medical condition involving an inflammation of the pancreas. In acute or chronic pancreatitis the inflammation manifests itself in the release and activation of pancreatic enzymes within the organ itself, leading to autodigestion. In many cases of
30 acute pancreatitis, the condition can lead to death.

In normal mammals, the pancreas, a large gland similar in structure to the salivary gland, is responsible for the production and secretion of digestive enzymes, which digest ingested food, and bicarbonate for the neutralization of the
35 acidic chyme produced in the stomach. The pancreas contains

5 acinar cells, responsible for enzyme production, and ductal
cells, which secrete large amounts of sodium bicarbonate
solution. The combined secretion product is termed
"pancreatic juice"; this liquid flows through the pancreatic
duct past the sphincter of Oddi into the duodenum. The
10 secretion of pancreatic juice is stimulated by the presence
of chyme in the upper portions of the small intestine, and
the precise composition of pancreatic juice appears to be
influenced by the types of compounds (carbohydrate, lipid,
protein, and/or nucleic acid) in the chyme.

15 The constituents of pancreatic juice includes proteases
(trypsin, chymotrypsin, carboxypolypeptidase), nucleases
(RNase and DNase), pancreatic amylase, and lipases
(pancreatic lipase, cholesterol esterase and phospholipase).

Many of these enzymes, including the proteases, are
20 initially synthesized by the acinar cells in an inactive
form as zymogens: thus trypsin is synthesized as
trypsinogen, chymotrypsin as chymotrypsinogen, and
carboxypolypeptidase as procarboxypolypeptidase. These
enzymes are activated according to a cascade, wherein, in
25 the first step, trypsin is activated through proteolytic
cleavage by the enzyme enterokinase. Trypsinogen can also
be autoactivated by trypsin; thus one activation has begun,
the activation process can proceed rapidly. Trypsin, in
turn, activates both chymotrypsinogen and
30 procarboxypolypeptidase to form their active protease
counterparts.

The enzymes are normally activated only when they enter
the intestinal mucosa in order to prevent autodigestion of
the pancreas. In order to prevent premature activation, the

5 acinar cells also co-secrete a trypsin inhibitor that normally prevents activation of the proteolytic enzymes within the secretory cells and in the ducts of the pancreas. Inhibition of trypsin activity also prevents activation of the other proteases.

10 Pancreatitis can occur when an excess amount of trypsin saturates the supply of trypsin inhibitor. This, in turn, can be caused by underproduction of trypsin inhibitor, or the overabundance of trypsin within the cells or ducts of the pancreas. In the latter case, pancreatic trauma or
15 blockage of a duct can lead to localized overabundance of trypsin; under acute conditions large amounts of pancreatic zymogen secretion can pool in the damaged areas of the pancreas. If even a small amount of free trypsin is available activation of all the zymogenic proteases rapidly
20 occurs, and can lead to digestion of the pancreas (acute pancreatitis) and in particularly severe cases to the patient's death.

Pancreatic secretion is normally regulated by both hormonal and nervous mechanisms. When the gastric phase of
25 stomach secretion occurs, parasympathetic nerve impulses are relayed to the pancreas, which initially results in acetylcholine release, followed by secretion of enzymes into the pancreatic acini for temporary storage.

When acid chyme thereafter enters the small intestine,
30 the mucosal cells of the upper intestine release a hormone called secretin. In humans, secretin is a 27 amino acid (3400 Dalton) polypeptide initially produced as the inactive form prosecretin, which is then activated by proteolytic cleavage. Secretin is then absorbed into the blood.

5 Secretin causes the pancreas to secrete large quantities of
a fluid containing bicarbonate ion. Secretin does not
stimulate the acinar cells, which produce the digestive
enzymes. The bicarbonate fluid serves to neutralize the
chyme and to provide a slightly alkaline optimal environment
10 for the enzymes.

Another peptide hormone, cholecystokinin (CCK) is
released by the mucosal cells in response to the presence of
food in the upper intestine. As described in further detail
below, human CCK is synthesized as a protoprotein of 115
15 amino acids. Active CCK forms are quickly taken into the
blood through the digestive tract, and normally stimulate
the secretion of enzymes by the acinar cells. However,
stimulation of the CCK receptor by the CCK analogs cerulein
and CCK-octapeptide (CCK-8) appears to lead to a worsening
20 of morbidity and mortality in mammals in whom pancreatitis
is induced. See Tani et al., *Pancreas* 5:284-290 (1990).

As indicated above, the digestive enzymes are
synthesized as zymogens; proto-enzyme synthesis occurs in
the rough endoplasmic reticulum of the acinar cells. The
25 zymogens are then packaged within vesicles having a single
lipid bilayer membrane. The zymogens are packed within the
vesicles so densely that they appear as quasi-crystalline
structures when observed under light microscopy and the
zymogen granules are electron-dense when observed under the
30 electron microscope. The vesicles are localized within the
cytoplasm of the acinar cells. Secretion of zymogens by the
acinar cells occurs through vesicle docking and subsequent
fusion with the plasma membrane, resulting in the liberation
of the contents into the extracellular milieu.

5 Nerve cells appear to secrete neurotransmitters
and other intercellular signaling factors through a
mechanism of membrane fusion that is shared with other cell
types, see e.g., Rizo & Sudhof, *Nature Struct. Biol.* 5:839-
842 (October 1998), hereby incorporated by reference herein,
10 including the pancreatic acinar cells.

Although the Applicants do not wish to be bound by
theory, it is believed that a vesicle first contacts the
intracellular surface of the cellular membrane in a reaction
called docking. Following the docking step the membrane
15 fuses with and becomes part of the plasma membrane through a
series of steps that currently remain relatively
uncharacterized, but which clearly involve certain vesicle
and membrane-associated proteins, as has been illustrated
using neural models.

20 In neurons, neurotransmitters are packaged within
synaptic vesicles, formed within the cytoplasm, then
transported to the inner plasma membrane where the vesicles
dock and fuse with the plasma membrane. Recent studies of
nerve cells employing clostridial neurotoxins as probes of
25 membrane fusion have revealed that fusion of synaptic
vesicles with the cell membrane in nerve cells depends upon
the presence of specific proteins that are associated with
either the vesicle or the target membrane. *See id.* These
proteins have been termed SNAREs. As discussed in further
30 detail below, a protein alternatively termed synaptobrevin
or VAMP (vesicle-associated membrane protein) is a vesicle-
associated SNARE (v-SNARE). There are at least two isoforms
of synaptobrevin; these two isoforms are differentially
expressed in the mammalian central nervous system, and are

5 selectively associated with synaptic vesicles in neurons and
secretory organelles in neuroendocrine cells. The target
membrane-associated SNAREs (t-SNAREs) include syntaxin and
SNAP-25. Following docking, the VAMP protein forms a core
complex with syntaxin and SNAP-25; the formation of the core
10 complex appears to be an essential step to membrane fusion.
See Rizo & Sudhof, *id.* and Neimmann et al., *Trends in Cell
Biol.* 4:179-185 (May 1994), hereby incorporated by
referenced herein.

15 Recently evidence has increasingly indicated that
the SNARE system first identified in neural cells is a
general model for membrane fusion in eukaryotic cells. A
yeast exocytotic core complex similar to that of the
synaptic vesicles of mammalian neural cells has been
characterized, and found to contain three proteins: Sso 1
20 (syntaxin 1 homolog), SncI (synaptobrevin homolog), and sec9
(SNAP-25 homolog). Rizo & Sudhof, *id.* These proteins share
a high degree of amino acid sequence homology with their
mammalian synaptosomal counterparts.

25 All mammalian non-neuronal cells appear to contain
cellubrevin, a synaptobrevin analog - this protein is
involved in the intracellular transport of vesicles, and is
cleaved by TeTx, BoNT/E, BoNT/F, and BoNT/G. Homologs of
syntaxin have been identified in yeast (e.g., ssolp and
sso2p) and mammalian non-neuronal cells (syn2p, syn3p, syn4p
30 and syn5p). Finally, as indicated above, a yeast SNAP-25
homolog, sec9 has been identified; this protein appears to
essential for vesicle fusion with the plasma membrane.

Intoxication of neural cells by clostridial
neurotoxins exploits specific characteristics of the SNARE

5 proteins. These neurotoxins, most commonly found expressed
in *Clostridium botulinum* and *Clostridium tetanus*, are highly
potent and specific poisons of neural cells. These Gram
positive bacteria secrete two related but distinct toxins,
each comprising two disulfide-linked amino acid chains: a
10 light chain (L) of about 50 KDa and a heavy chain (H) of
about 100 KDa, which are wholly responsible for the symptoms
of botulism and tetanus, respectively.

The tetanus and botulinum toxins are among the most
lethal substances known to man; both toxins function by
15 inhibiting neurotransmitter release in affected neurons.
The tetanus neurotoxin (TeNT) acts mainly in the central
nervous system, while botulinum neurotoxin (BoNT) acts at
the neuromuscular junction; both toxins inhibit
acetylcholine release from the nerve terminal of the
20 affected neuron into the synapse, resulting in paralysis or
reduced target organ function.

The tetanus neurotoxin (TeNT) is known to exist in one
immunologically distinct type; the botulinum neurotoxins
(BoNT) are known to occur in seven different immunologically
25 distinct serotypes, termed BoNT/A through BoNT/G. While all
of these latter types are produced by isolates of *C.*
botulinum, two other species, *C. baratii* and *C. butyricum*
also produce toxins similar to /F and /E, respectively. See
e.g., Coffield et al., *The Site and Mechanism of Action of*
30 *Botulinum Neurotoxin in Therapy with Botulinum Toxin 3-13*
(Jankovic J. & Hallett M. eds. 1994), the disclosure of
which is incorporated herein by reference.

Regardless of type, the molecular mechanism of
intoxication appears to be similar. In the first step of

5 the process, the toxin binds to the presynaptic membrane of
the target neuron through a specific interaction between the
heavy chain and a neuronal cell surface receptor; the
receptor is thought to be different for each type of
botulinum toxin and for TeNT. The carboxy terminal (C-
10 terminal) half of the heavy chain is required for targeting
of the toxin to the cell surface. The cell surface
receptors, while not yet conclusively identified, appear to
be distinct for each neurotoxin serotype.

In the second step, the toxin crosses the plasma
15 membrane of the poisoned cell. The toxin is first engulfed
by the cell through receptor-mediated endocytosis, and an
endosome containing the toxin is formed. The toxin (or
light chain thereof) then escapes the endosome into the
cytoplasm of the cell. This last step is thought to be
20 mediated by the amino terminal (N-terminal) half of the
heavy chain, which triggers a conformational change of the
toxin in response to a pH of about 5.5 or lower. Endosomes
are known to possess a proton pump that decreases intra-
endosomal pH. The conformational shift exposes hydrophobic
25 residues in the toxin, which permits the toxin to embed
itself in the endosomal membrane. The toxin then
translocates through the endosomal membrane into the
cytosol.

Either during or after translocation the disulfide bond
30 joining the heavy and light chain is reduced, and the light
chain is released into the cytoplasm. The entire toxic
activity of botulinum and tetanus toxins is contained in the
light chain of the holotoxin; the light chain is a zinc
(Zn++) endopeptidase which selectively cleaves the SNARE

5 proteins essential for recognition and docking of
neurotransmitter-containing vesicles with the cytoplasmic
surface of the plasma membrane, and fusion of the vesicles
with the plasma membrane. The light chain of TxNT, BoNT/B,
BoNT/D, BoNT/F, and BoNT/G cause specific proteolysis of
10 VAMP, an integral protein. During proteolysis, most of the
VAMP present at the cytosolic surface of the synaptic
vesicle is inactivated as a result of any one of these
cleavage events. Each toxin cleaves a different specific
peptide bond.

15 BoNT/A and /E selectively cleave the plasma membrane-
associated SNARE protein SNAP-25; this protein is bound to
and present on the cytoplasmic surface of the plasma
membrane. BoNT/C1 cleaves syntaxin, which exists as an
integral protein having most of its mass exposed to the
20 cytosol. Syntaxin interacts with the calcium channels at
presynaptic terminal active zones. See Tonello et al.,
*Tetanus and Botulism Neurotoxins in Intracellular Protein
Catabolism* 251-260 (Suzuki K & Bond J. eds. 1996), the
disclosure of which is incorporated by reference as part of
25 this specification. Bo/NTC1 also appears to cleave SNAP-25.

Both TeNT and BoNT are specifically taken up by cells
present at the neuromuscular junction. BoNT remains within
peripheral neurons and, as indicated above, blocks release
of the neurotransmitter acetylcholine from these cells.

30 By contrast TeNT, through its receptor, enters vesicles
that move in a retrograde manner along the axon to the soma,
and is discharged into the intersynaptic space between motor
neurons and the inhibitory neurons of the spinal cord. At
this point, TeNT binds receptors of the inhibitory neurons,

5 is again internalized, and the light chain enters the
cytosol to block the release of the inhibitory
neurotransmitters 4-aminobutyric acid (GABA) and glycine
from these cells. Id.

International Patent Publication No. WO 96/33273
10 relates to derivatives of botulinum toxin designed to
prevent neurotransmitter release from sensory afferent
neurons to treat chronic pain. Such derivatives are
targeted to nociceptive neurons using a targeting moiety
that binds to a binding site of the surface of the neuron.

15 International Patent Publication No. 98/07864 discusses
the production of recombinant toxin fragments that have
domains that enable the polypeptide to translocate into a
target cell or which increase the solubility of the
polypeptide, or both.

20

Summary of the Invention

The present invention concerns methods and compositions
25 useful for the treatment of acute pancreatitis. This
condition is largely due to the defective secretion of
zymogen granules by acinar cells, and by the premature co-
mingling of the secreted zymogens with lysosomal
hydrolysates capable of activating trypsin, thereby
30 triggering the protease activation cascade and resulting in
the destruction of pancreatic tissue.

In one embodiment of this aspect, the invention is a
therapeutic agent comprising a chimeric protein containing
an amino acid sequence-specific endopeptidase activity which

5 will specifically cleave at least one synaptic vesicle-
associated protein selected from the group consisting of
SNAP-25, syntaxin or VAMP, in combination with the
translocation activity of the N-terminus of a clostridial
neurotoxin heavy chain, wherein the chimeric protein further
10 comprises a recognition domain which will bind a human
cholecystokinin (CCK) receptor. Upon binding of the
recognition domain of the protein to the CCK receptor, the
protein is specifically transported into cells containing
CCK receptors (pancreatic acinar cells) through receptor-
15 mediated endocytosis. In a preferred embodiment, the CCK
receptor is the CCK A receptor.

Once inside the acinar cell, the chimeric protein
functions in a manner similar to that of a clostridial
neurotoxin within its target neuron. The toxin moiety is
20 translocated from the endosome into the cytoplasm, where it
acts to cleave a SNARE protein identical or homologous to
SNAP-25, syntaxin or VAMP. The cleavage of this protein
prevents formation of a core complex between the SNARE
proteins and thus prevents or reduces the extent of fusion
25 of the vesicle with the target membrane. This, in turn,
results in inhibition of zymogen release from the acinar
cells and of zymogen activation by lysosomal hydrolases.
The autodigestion of pancreatic tissue in acute pancreatitis
is therefore reduced or eliminated.

30 Another embodiment of the present invention concerns a
method of treating a patient suffering from acute
pancreatitis by administering an effective amount of such a
chimeric protein.

Another embodiment of the invention concerns a

5 therapeutic composition that contains the translocation
activity of a clostridial neurotoxin heavy chain in
combination with a recognition domain able to bind a
specific cell type and a therapeutic element having an
activity other than the endopeptidase activity of a
10 clostridial neurotoxin light chain. A non-exclusive list of
certain such therapeutic elements includes: hormones and
hormone-agonists and antagonists, nucleic acids capable
being of being used as replication, transcription, or
translational templates (e.g., for expression of a protein
15 drug having the desired biological activity or for synthesis
of a nucleic acid drug as an antisense agent), enzymes,
toxins, and the like.

In a preferred embodiment, the specific cell type is a
pancreatic cell, most preferably a pancreatic acinar cell.

20 Another embodiment is drawn to methods for the
treatment of acute pancreatitis comprising contacting an
acinar cell with an effective amount of a composition
comprising a chimeric protein containing an amino acid
sequence-specific endopeptidase activity which will
25 specifically cleave at least one synaptic vesicle-associated
protein selected from the group consisting of SNAP-25,
syntaxin or VAMP, in combination with the translocation
activity of the N-terminus of a clostridial neurotoxin heavy
chain, wherein the chimeric protein further comprises a
30 recognition domain able to bind to a cell surface protein
characteristic of an human pancreatic acinar cell.
Preferably the cell surface protein is a CCK receptor
protein; most preferably the protein is the human CCK A
protein. CCK receptors (CCK-A receptor and CCK-B receptor)

5 are found mainly in on the surface of pancreatic acinar cells, although they are also found in some brain cells and, to a lesser extent on the surface of gastrointestinal cells.

Any suitable route of administration may be used in this aspect of the invention. Applicants currently prefer
10 to administer the therapeutic agent in an intravenous infusion solution; however methods such as ingestion (particularly when associated with neurotoxin-associated proteins (NAPs); see Sharma et al., *J. Nat. Toxins* 7:239-253(1998), incorporated by reference herein), direct
15 delivery to the pancreas, injection and the like may also be used. The agent is substantially specifically targeted to pancreatic cells; when the agent contains a CCK receptor-binding domain, the blood-brain barrier prevents the agent from interacting with brain cells.

20 In yet another embodiment the invention provides a composition comprising a drug or other therapeutic agent having an activity other than that of a clostridial neurotoxin light chain for intracellular delivery, said agent joined to the translocation domain of a clostridial
25 neurotoxin heavy chain and a binding element able to recognize a cell surface receptor of a target cell. In a preferred embodiment, the target cell is not a neuron. Also, in this embodiment it is preferred that the drug or other therapeutic agent has an enzymatic, catalytic, or
30 other self-perpetuating mode of activity, so that the effective dose of drug is greater than the number of drug molecules delivered within the target cell. A non-exclusive list of certain such drugs would include: hormones and hormone-agonists and antagonists, nucleic acids capable

5 being of being used as replication, transcription, or translational templates (e.g., for expression of a protein drug having the desired biological activity or for synthesis of a nucleic acid drug as an antisense agent), enzymes, toxins (such as diphtheria toxin or ricin), and the like.

10 In this embodiment the drug may be cleavably linked to the remainder of the composition in such a way as to allow for the release of the drug from the composition within the target cell.

The presently claimed compositions may be provided to 15 the patient by intravenous administration, may be administered during surgery, or may be provided parenterally.

WO 95/32738, which is shares ownership with the present application, describes transport proteins for the 20 therapeutic treatment of neural cells. This application is incorporated by reference herein as part of this specification.

Detailed Description of the Preferred Embodiments

25 In a basic and presently preferred form, the invention comprises a therapeutic polypeptide comprising three features: a binding element, a translocation element, and a therapeutic element.

30 The binding element is able to bind to a specific target cell provided that the target cell is not a motor neuron or a sensory afferent neuron. Preferably, the binding element comprises an amino acid chain; also an independently, it is preferably located at or near the C-

5 terminus of a polypeptide chain. By "binding element" is
meant a chemical moiety able to preferentially bind to a
cell surface marker characteristic of the target cell under
physiological conditions. The cell surface marker may
comprise a polypeptide, a polysaccharide, a lipid, a
10 glycoprotein, a lipoprotein, or may have structural
characteristics of more than one of these. By
"preferentially interact" is meant that the disassociation
constant (K_d) of the binding element for the cell surface
marker is at least one order of magnitude less than that of
15 the binding element for any other cell surface marker.
Preferably, the disassociation constant is at least 2 orders
of magnitude less, even more preferably the disassociation
constant is at least 3 orders of magnitude less than that of
the binding element for any other cell surface marker to
20 which the therapeutic polypeptide is exposed. Preferably,
the organism to be treated is a human.

In one embodiment the cell surface receptor comprises
the histamine receptor, and the binding element comprises an
variable region of an antibody which will specifically bind
25 the histamine receptor.

In an especially preferred embodiment, the cell surface
marker is a cholecystokinin (CCK) receptor. Cholecystokinin
is a bioactive peptide that functions as both a hormone and
a neurotransmitter in a wide variety of physiological
30 settings. Thus, CCK is involved in the regulation of gall
bladder contraction, satiety, gastric emptying, and gut
motility; additionally it is involved in the regulation of
pancreatic exocrine secretion.

5 There are two types of CCK receptors, CCK A and CCK B;
the amino acid sequences of these receptors have been
determined from cloned cDNA. Despite the fact that both
receptors are G protein-coupled receptors and share
approximately 50% homology, there are distinct differences
10 between their physiological activity. The CCK A receptor is
expressed in smooth muscle cells of the gall bladder, smooth
muscle and neurons within the gastrointestinal tract, and
has a much greater affinity ($>10^2$ times higher) for CCK than
the related peptide hormone gastrin. The CCK B receptor,
15 found in the stomach and throughout the CNS, has roughly
equal ability to bind CCK and gastrin.

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The varied activities of CCK can be partly attributed
to the fact that CCK is synthesized as procholecystokinin, a
protoprotein of 115 amino acids, and is then post-
20 translationally cleaved into a number of active fragments
all sharing the same C-terminus. The amino acid sequence of
human procholecystokinin is shown below; amino acid residues
not present in the biologically active cleavage products are
in lower case. All amino acid sequences herein are shown
25 from N-terminus to C-terminus, unless expressly indicated
otherwise:

Human procholecystokinin, having the amino acid
sequence SEQ ID NO:1:

30

mmsgvclcvlmavlaagaltqpvp padpagsglqraeeaprrqlr VSQRT
DGESRAHLGA LLARYIQQAR KAPSGRMSIV KNLQNLDPSH RISDRDYMGW
MDF grrsaeeyeyps

5 Biologically active cleavage products of the full
length CCK chain include:

CCK-58, having the amino acid sequence SEQ ID NO:2:

VSQRT DGESRAHLGA LLARYIQQAR KAPSGRMSIV KNLQNLDP SH
10 RISDRDYMGW MDF;

CCK-39, having the amino acid sequence SEQ ID NO: 3:

YIQQAR KAPSGRMSIV KNLQNLDP SH RISDRDYMGW MDF;
15

CCK-33, having the amino acid sequence SEQ ID NO: 4:

KAPSGRMSIV KNLQNLDP SH RISDRDYMGW MDF;

20 CCK-12, having the amino acid sequence SEQ ID NO: 5:

ISDRDYMGW MDF;

and CCK-8, having the amino acid sequence SEQ ID NO: 6:

25 RDYMGW MDF.

In each case, the biologically active polypeptides
contain two additional post-translational modifications;
amidation of the C-terminal phenylalanine, and sulfatation
30 of the aspartic acid residue located seven residue from the
C-terminus of the biologically active species. These
modifications appear to be necessary for full biological
activity, although both the C-terminal pentapeptide and
tetrapeptide of CCK retains some biological activity.

- 5 Kennedy et al., *J. Biol. Chem.* 272: 2920-2926 (1997), hereby incorporated by reference herein.

While it will be understood that the applicants do not wish to be bound by theory, the following findings may assist an understanding the nature of the interaction
10 between CCK and the CCK receptors, and thus between the CCK receptor binding element of an embodiment of the present invention and its CCK receptor target.

In pancreatic acinar cells the CCK A receptor undergoes internalization to intracellular sites within minutes after
15 agonist exposure. Pohl et al., *J. Biol. Chem.* 272: 18179-18184 (1997), hereby incorporated by reference herein. The CCK B receptor has also shown the same ligand-dependant internalization response in transfected NIH 3T3 cells. In the CCK B receptor, but not the CCK A receptor, the
20 endocytotic feature of the receptor been shown to be profoundly decreased by the deletion of the C terminal 44 amino acids of the receptor chain, corresponding in both receptors to an cytoplasmic portion of the receptor chain.

Recent studies of the interaction between the CCK A
25 receptor and CCK have shown that the primary receptor sequence region containing amino acid residues 38 through 42 is involved in the binding of CCK. Residues Trp₃₉ and Gln₄₀ appear to be essential for the binding of a synthetic CCK C-terminal nonapeptide (in which the methionine residues
30 located at residue 3 and 6 from the C-terminus are substituted by norleucine and threonine respectively) to the receptor. Kennedy et al., *supra*. These residues do not appear to be essential for the binding of CCK analogs JMV 180 (corresponding the synthetic C-terminal heptapeptide of

5 CCK in which the phenylalanylamide residue is substituted by
a phenylethyl ester and the threonine is substituted with
norleucine), and JMV 179 (in which the phenylalanylamide
residue and the L-tryptophan residues of the synthetic CCK
nonapeptide are substituted by a phenylethyl ester and D-
10 tryptophan, respectively and the threonine is substituted
with norleucine). *Id.*

These and similar studies have shed light on the
structure of the CCK A receptor active site. Based on
receptor binding experiments, a current structural model
15 indicates that CCK residues Trp₃₀ and Met₃₁ (located at
positions 4 and 3, respectively, from the C terminus of
mature CCK-8) reside in a hydrophobic pocket formed by
receptor residues Leu₃₄₈, Pro₃₅₂, Ile₃₅₃ and Ile₃₅₆. CCK residue
Asp₃₂ (located at amino acid position 2 measured from the C
20 terminus of CCK-8) seems to be involved in an ionic
interaction with receptor residue Lys₁₁₅. CCK Tyr-sulfate₂₇
(the CCK-8 residue 7 amino acids from C terminus) appears
involved in an ionic interaction with receptor residue Lys₁₀₅
and a stacking interaction with receptor residue Phe₁₉₈. Ji,
25 et al., 272 *J. Biol. Chem.* 24393-24401 (1997).

Such structural models provide detailed guidance to the
person of ordinary skill in the art as to the construction
of a variety of binding elements able to retain the binding
characteristics of biologically active CCK peptides for the
30 CCK-A receptor, for example, as, for example, by site
directed mutagenesis of a clostridial neurotoxin heavy
chain. Similarly, models deduced using similar methodologies
have been proposed for the CCK B receptor, see e.g.,

5 Jagerschmidt, A. et al., *Mol. Pharmacol.* 48:783-789 (1995),
and can be used as a basis for the construction of binding
elements that retain binding characteristics similar to the
CCK B receptor.

10 Additionally, the binding element may comprise a
variable region of an antibody which will bind the CCK-A or
CCK-B receptor.

Nucleic acids encoding polypeptides containing such a
binding element may be constructed using molecular biology
methods well known in the art; see e.g., Sambrook et al.,
15 *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor
Laboratory Press 2d ed. 1989), and expressed within a
suitable host cell. The disclosure of this latter reference
is incorporated by reference herein in its entirety.

20 The translocation element comprises a portion of a
clostridial neurotoxin heavy chain having a translocation
activity. By "translocation" is meant the ability to
facilitate the transport of a polypeptide through a
vesicular membrane, thereby exposing some or all of the
polypeptide to the cytoplasm.

25 In the various botulinum neurotoxins translocation is
thought to involve an allosteric conformational change of
the heavy chain caused by a decrease in pH within the
endosome.

30 This conformational change appears to involve and be
mediated by the N terminal half of the heavy chain and to
result in the formation of pores in the vesicular membrane;
this change permits the movement of the proteolytic light
chain from within the endosomal vesicle into the cytoplasm.

5 See e.g., Lacy, et al., *Nature Struct. Biol.* 5:898-902
(October 1998).

The amino acid sequence of the translocation-mediating
portion of the botulinum neurotoxin heavy chain is known to
those of skill in the art; additionally, those amino acid
10 residues within this portion that are known to be essential
for conferring the translocation activity are also known.

It would therefore be well within the ability of one of
ordinary skill in the art, for example, to employ the
naturally occurring N-terminal peptide half of the heavy
15 chain of any of the various *Clostridium tetanus* or
Clostridium botulinum neurotoxin subtypes as a translocation
element, or to design an analogous translocation element by
aligning the primary sequences of the N-terminal halves of
the various heavy chains and selecting a consensus primary
20 translocation sequence based on conserved amino acid,
polarity, steric and hydrophobicity characteristics between
the sequences.

The therapeutic element of the present invention may
comprise, without limitation: active or inactive (i.e.,
25 modified) hormone receptors (such as androgen, estrogen,
retinoid, peroxisome proliferator and ecdysone receptors
etc.), and hormone-agonists and antagonists, nucleic acids
capable of being used as replication, transcription,
or translational templates (e.g., for expression of a
30 protein drug having the desired biological activity or for
synthesis of a nucleic acid drug as an antisense agent),
enzymes, toxins (including apoptosis-inducing agents), and
the like.

5 In a preferred embodiment, the therapeutic element is a polypeptide comprising a clostridial neurotoxin light chain or a portion thereof retaining the SNARE-protein sequence-specific endopeptidase activity of a clostridial neurotoxin light chain. The amino acid sequences of the light chain of
10 botulinum neurotoxin (BoNT) subtypes A-G have been determined, as has the amino acid sequence of the light chain of the tetanus neurotoxin (TeNT). Each chain contains the Zn⁺⁺-binding motif **His-Glu-x-x-His** (N terminal direction at the left) characteristic of Zn⁺⁺-dependent endopeptidases
15 (HELIH in TeNT, BoNT/A /B and /E; HELNH in BoNT/C; and HELTH in BoNT/D).

Recent studies of the BoNT/A light chain have revealed certain features important for the activity and specificity of the toxin towards its target substrate, SNAP-25. Thus,
20 studies by Zhou et al. *Biochemistry* 34:15175-15181 (1995) have indicated that when the light chain amino acid residue His₂₂₇ is substituted with tyrosine, the resulting
A polypeptide is unable to cleave SNAP-25; ^{Kurazono}~~Kurazono~~ et al., *J. Biol. Chem.* 14721-14729 (1992) performed studies in the
25 presynaptic cholinergic neurons of the buccal ganglia of *Aplysia californica* using recombinant BoNT/A light chain that indicated that the removal of 10 N-terminal or 32 C-terminal residues did not abolish toxicity, but that removal of 10 N-terminal or 57 C-terminal residues abolished
30 toxicity in this system. Most recently, the crystal structure of the entire BoNT/A holotoxin has been solved; the active site is indicated as involving the participation of His₂₂₂, Glu₂₂₃, His₂₂₆, Glu₂₆₁ and Tyr₃₆₅. Lacy et al., *supra*.

5 (These residues correspond to His²²³, Glu²²⁴, His²²⁷, Glu²⁶² and
Tyr³⁶⁶ of the BoNT/A L chain of Kurazono et al., *supra*.)
Interestingly, an alignment of BoNT/A through E and TeNT
light chains reveals that every such chain invariably has
these residues in positions analogous to BoNT/A. Kurazono
10 et al., *supra*.

The catalytic domain of BoNT/A is very specific for the
C-terminus of SNAP-25 and appears to require a minimum of 16
SNAP-25 amino acids for cleavage to occur. The catalytic
site resembles a pocket; when the light chain is linked to
15 the heavy chain via the disulfide bond between Cys⁴²⁹ and
Cys⁴⁵³, the translocation domain of the heavy chain appears
to block access to the catalytic pocket until the light
chain gains entry to the cytosol. When the disulfide bond
is reduced, the two polypeptide chains dissociate, and the
20 catalytic pocket is then "opened" and the light chain is
fully active.

As described above, VAMP and syntaxin are cleaved by
BoNT/B, D, F, G and TeNT, and BoNT/C₁, respectively, while
SNAP-25 is cleaved by BoNT/A and E.

25 The substrate specificities of the various clostridial
neurotoxin light chains other than BoNT/A are known.
Therefore, the person of ordinary skill in the art could
easily determine the toxin residues essential in these
subtypes for cleavage and substrate recognition (for
30 example, by site-directed mutagenesis or deletion of various
regions of the toxin molecule followed by testing of
proteolytic activity and substrate specificity), and could

5 therefore easily design variants of the native neurotoxin
light chain that retain the same or similar activity.

Additionally, construction of the therapeutic agents
set forth in this specification would be easily constructed
by the person of skill in the art. It is well known that the
10 clostridial neurotoxins have three functional domains
analogous to the three elements of the present invention.
For example, the BoNT/A neurotoxin light chain is present in
amino acid residues 1-448 of the BoNT/A prototoxin (i.e.,
before nicking of the prototoxin to form the disulfide-
15 linked dichain holotoxin); this amino acid sequence is
provided below as SEQ ID NO: 7. Active site residues are
underlined:

BoNT/A light chain (SEQ ID NO:7)

20

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNKIWV
IPERDTFTNP EEGDLNPPPEAKQVPVSYDSTYLSTDNEKDNYLKGVTKLFERIYSTD
LGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADI
IQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLL GAGKFATDPA
25 VTLAHELIHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHD A K F I D S
LQENEFRLYYNKF KD IASTLNKAKSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVD
KLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKINIVPKVNYTIYDGFNL
RNTNLAANFNGQNT EINN MNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNK;

30 The heavy chain N-terminal (HN) translocation domain is
contained in amino acid residues 449-871 of the BoNT/A amino
acid sequence, shown below as SEQ ID NO: 8; a gated ion
channel-forming domain probably essential for the
translocation activity of this peptide is underlined (see
35 Oblatt-Montal et al., *Protein Sci.* 4:1490-1497(1995), hereby
incorporated by reference herein.

5 ALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNF
 DNEPENISIENLSSDIIGQLELMPNIEFPPNGKKYELDKYTMFHYLRAQEFEGHKSRI
 ALTNSVNEALLNPSRVYTFSSDYVKKVKNKATEAAMFLGWVEQLVYDFTDETSEVSTT
 DKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALV
 SYIANKVLTQVQIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQA
 10 EATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMN
 SMIPYGVKRLDFDASLKDALLKYIYDNRGTLIGQVDRLKDKVNNTLSTDIPFQLSKY
 VDNQRLSTFTTEYIK;

The heavy chain C-terminal neural cell binding domain
 15 is contained in amino acid residues 872-1296 (SEQ ID NO: 9)
 of the BoNT/A prototoxin.

NIINTSILNRLRYESNHLIDLSRYASKINIGSKVNFDPIDKNQI
 QLFNLESSKIEVILKNAIVYNSMYENFSTSFWIRIPKYFNSISLNNEYTIINCMENNS
 20 GWKVSLNYGEIIWTLQDTQEIKQRVVFKYSQMINISDYINRWIFVTITNNRLNNSKIY
 INGRLLIDQKPISNLGNIHASNNIMFKLDGCRDTHRYIWIKYFNLFDKELNEKEIKDLY
 DNQSNISGILKDFWGDYLDYDKPYMLNLYDPNKYVDVNVGIRGYMYLKGPRGSVMTT
 NIYLNSSLYRGTKFIKKYASGNKDNIVRNNDRVYINVVVKNKEYRLATNASQAGVEK
 ILSALEIPDVGNLSQVVMKSKNDQGITNKCKMNLQDNNGNDIGFIGFHQFNNAIKLV
 25 ASNWYNRQIERSSRTLGCSEFIPVDDGWGERPL

The amino acid sequence of the BoNT/A prototoxin is encoded
 by nucleotides 358 to 4245 of the neurotoxin cDNA sequence,
 set forth herein below as SEQ ID NO: 10.

30 aagcttctaa atttaaatta ttaagtataa atccaaataa acaatatggt caaaaacttg
 atgaggtaat aatttctgta ttagataata tggaaaaata tatagatata tctgaagata
 atagattgca actaatagat acaaaaaata acgcaaagaa gatgataatt agtaatgata
 tatttatttc caattgttta accctatctt ataacggtaa atatatatgt ttatctatga
 35 aagatgaaaa ccataattgg atgatatgta ataatgatat gtcaaagtat ttgtatttat
 ggtcatttaa ataattaata atttaattaa ttttaaatat tataagaggt gttaaatatg
 ccatttggtta ataaacaatt taattataaa gatcctgtaa atggtgttga tattgcttat
 ataaaaattc caaatgcagg acaaatgcaa ccagtaaaag cttttaaaat tcataataaa
 atatgggtta ttccagaaag agatacattt acaaatcctg aagaaggaga tttaaattcca
 40 ccaccagaag caaaacaagt tccagtttca tattatgatt caacatattt aagtacagat
 aatgaaaaag ataattattt aaaggagggt acaaaattat ttgagagaat ttattcaact
 gatcttgga gaattgtgtt aacatcaata gtaaggggaa taccattttg ggggtggaagt
 acaatagata cagaattaaa agttattgat actaattgta ttaatgtgat acaaccagat
 ggtagttata gatcagaaga acttaattcta gtaataatag gaccctcagc tgatattata
 45 cagtttgaat gtaaaagctt tggacatgaa gttttgaatc ttacgcgaaa tgggttatggc
 tctactcaat acattagatt tagcccagat tttacatttg gttttgagga gtcacttgaa

5 gttgatacaa atcctctttt aggtgcaggc aaatttgcta cagatccagc agtaacatta
gcacatgaac ttatacatgc tggacataga ttatatggaa tagcaattaa tccaaatagg
gttttttaaag taaatactaa tgcctattat gaaatgagtg ggtagaagt aagctttgag
gaacttagaa catttggggg acatgatgca aagtttatag atagtttaca ggaaaacgaa
tttcgtctat attattataa taagttttaa gatatagcaa gtacacttaa taaagctaaa
10 tcaatagtag gtactactgc ttcattacag tatatgaaaa atgtttttta agagaaatat
ctcctatctg aagatacatc tggaaaattt tcggtagata aattaaaatt tgataagtta
tacaaaatgt taacagagat ttacacagag gataattttg ttaagttttt taaagtactt
aacagaaaaa catatttgaa ttttgataaa gccgtattta agataaatat agtacctaag
gtaaattaca caatatatga tggattttaat ttaagaaata caaatttagc agcaaacttt
15 aatggtcaaa atacagaaat taataatatg aattttacta aactaaaaaa ttttactgga
ttgtttgaat tttataagtt gctatgtgta agagggataa taacttctaa aactaaatca
ttagataaag gatacaataa ggcattaaat gatttatgta tcaaagttta taattgggac
ttgtttttta gtccttcaga agataatttt actaatgatc taaataaagg agaagaaatt
acatctgata ctaatataga agcagcagaa gaaaatatta gtttagattt aatacaacaa
20 tattatttaa cctttaattt tgataatgaa cctgaaaata tttcaataga aaatctttca
agtacatta taggccaatt agaacttatg cctaatatag aaagatttcc taatggaaaa
aagtatgagt tagataaata tactatgttc cattatcttc gtgctcaaga atttgaacat
ggtaaactca ggattgcttt aacaaattct gttaacgaag cattattaaa tcctagtcgt
gtttatacat tttttcttc agactatgta aagaaagtta ataaagctac ggaggcagct
25 atgttttttag gctgggtaga acaattagta tatgatttta ccgatgaaac tagcgaagta
agtactacgg ataaaattgc ggatataact ataattattc catatatagg acctgcttta
aatataggta atatgttata taaagatgat tttgtaggtg ctttaatat ttcaggagct
gttattctgt tagaatttat accagagatt gcaataacctg tattaggtac ttttgcactt
gtatcatata ttgcgaataa ggttctaacc gttcaaacia tagataatgc ttttaagtaa
30 agaaatgaaa aatgggatga ggtctataaa tatatagtaa caaattgggt agcaaagggt
aatacacaga ttgatcta atagaaaaaa atgaaagaag ctttagaaaa tcaagcagaa
gcaacaaagg ctataataaa ctatcagtat aatcaatata ctgaggaaga gaaaaataat
attaatttta atattgatga ttttaagttcg aaacttaatg agtctataaa taaagctatg
attaatataa ataaattttt gaatcaatgc tctgtttcat atttaatgaa ttctatgatc
35 ccttatggtg ttaaacgggt agaagatttt gatgctagtc ttaaagatgc attattaaag
tatatatatg ataatagagg aactttaatt ggtcaagtag atagattaaa agataaagtt
aataatacac ttagtacaga tatacctttt cagctttcca aatacgtaga taatcaaaga
ttattatcta catttactga atatatata aatattatta atacttctat attgaattta
agatatgaaa gtaatcattt aatagactta tctagggtat catcaaaaat aaatattggt
40 agtaaagtaa attttgatcc aatagataaa aatcaaattc aattatttaa tttagaaagt
agtaaaattg aggtaatttt aaaaaatgct attgtatata atagtatgta tgaaaatttt
agtactagct tttggataag aattccctaag tattttaaca gtataagttt aaataatgaa
tatacaataa taaattgtat ggaaaataat tcaggatgga aagtatcact taattatggt
gaaataatct ggactttaca ggatactcag gaaataaaaac aaagagtagt ttttaaatac
45 agtcaaataga ttaatatatc agattatata aacagatgga tttttgtaac tatcactaat
aatagattaa ataactctaa aatttatata aatggaagat taatagatca aaaaccaatt
tcaaatttag gtaatatcca tgctagtaat aatataatgt ttaaattaga tggttgtaga
gatacacata gatatatattg gataaaatat tttaatcttt ttgataagga attaaatgaa
aaagaaatca aagatttata tgataatcaa tcaaattcag gtatttttaa agacttttgg
50 ggtgattatt tacaatatga taaaccatac tatatgttaa atttatatga tccaaataaa
tatgtcgatg taaataatgt aggtattaga gggttatatgt atcttaaagg gcctagaggt
agcgtaatga ctacaaacat ttattttaat tcaagtttgt atagggggac aaaatttatt
ataaaaaaat atgcttctgg aaataaagat aatattgtta gaaataatga tcgtgtatat

5 attaatgtag tagttaaaaa taaagaatat aggttagcta ctaatgcatc acaggcaggc
 gtagaaaaaa tactaagtgc attagaaata cctgatgtag gaaatctaag tcaagtagta
 gtaatgaagt caaaaaatga tcaaggaata acaaataaat gcaaaatgaa tttacaagat
 aataatggga atgatatagg ctttatagga tttcatcagt ttaataatat agctaaacta
 gtagcaagta attggtataa tagacaaata gaaagatcta gtaggacttt gggttgctca
 10 tgggaaattta ttcctgtaga tgatggatgg ggagaaaggc cactgtaatt aatctcaaac
 tacatgagtc tgtcaagaat tttctgtaaa catccataaa aatttttaaaa ttaatatgtt
 taagaataac tagatatgag tattgtttga actgcccctg tcaagtagac aggtaaaaaa
 ataaaaatta agatactatg gtctgatttc gatattctat cggagtcaga ccttttaact
 tttcttgat cctttttgta ttgtaaaact ctatgtattc atcaattgca agttccaatt
 15 agtcaaaatt atgaaacttt ctaagataat acatttctga ttttataatt tcccaaaatc
 cttccatagg accattatca atacatctac caactcgaga catactttga gttgcgccta
 tctcattaag tttattcttg aaagatttac ttgtatattg aaaaccgcta tcaactgtgaa
 aaagtggact agcatcagga ttggaggtaa ctgctttatc aaaggtttca aagacaagga
 cgttgttatt tgattttcca agtacatagg aaataatgct attatcatgc aaatcaagta
 20 tttactcaa gtacgccttt gtttcgtctg ttaac

Of course, three distinct domains analogous to those
 described above for BoNT/A exist for all the BoNT subtypes
 as well as for TeNT neurotoxin; an alignment of the amino
 25 acid sequences of these holotoxins will reveal the sequence
 coordinates for these other neurotoxin species.

Preferably, the translocation element and the binding
 element of the compositions of the present invention are
 separated by a spacer moiety that facilitates the binding
 30 element's binding to the desired cell surface receptor. Such
 a spacer may comprise, for example, a portion of the BoNT Hc
 sequence (so long as the portion does not retain the ability
 to bind to motor neurons or sensory afferent neurons),
 another sequence of amino acids, or a hydrocarbon moiety.

35 The spacer moiety may also comprise a proline, serine,
 threonine and/or cysteine-rich amino acid sequence similar
 or identical to a human immunoglobulin hinge region. In a
 preferred embodiment, the spacer region comprises the amino
 acid sequence of an immunoglobulin $\gamma 1$ hinge region; such a
 40 sequence has the sequence (from N terminus to C terminus):

5 EPKSCDKTHTCPPCP (SEQ ID NO:11)

It will be understood that none of the examples or embodiments described herein are to be construed as limiting the scope of the invention, which is defined solely by the claims that conclude this specification.

10

Example 1:

An agent for the treatment of acute pancreatitis is constructed as follows.

15

A culture of *Clostridium botulinum* is permitted to grown to confluence. The cells are then lysed and total RNA is extracted according to conventional methods and in the presence of an RNase inhibitor. The RNA preparation is then passed over a oligo(dT) cellulose column, the polyadenylated messenger RNA is permitted to bind, and the column is washed with 5-10 column volumes of 20 mM Tris pH 7.6, 0.5 M NaCl, 1 mM EDTA (ethylenediamine tetraacetic acid), 0.1% (w/v) SDS (sodium dodecyl sulfate). Polyadenylated RNA is then eluted with 2-3 column volumes of STE (10 mM Tris (pH 7.6), 1 mM EDTA, 0.05% (w/v) SDS). The pooled mRNA is then precipitated in 2 volumes of ice cold ethanol, pelleted in a centrifuge at 10,000 x g for 15 minutes, then redissolved in a small volume of STE.

The BoNT/A mRNA is used as a template for DNA synthesis using Moloney murine leukemia virus reverse transcriptase (MMLV-RT), then the L chain and then H_N chain of the neurotoxin is amplified from the cDNA by the polymerase chain reaction (PCR) using appropriate oligonucleotide primers whose sequences are designed based on the BoNT/A

5 neurotoxin cDNA sequence of SEQ ID NO: 9. These procedures
are performed using the standard techniques of molecular
biology as detailed in, for example, Sambrook et al.,
already incorporated by reference herein. The primer
defining the beginning of the coding region (5' side of the L
10 chain fragment) is given a StuI site. The PCR primer
defining the 3' end of the HN-encoding domain has the
following features (from 3' to 5'): a 5' region sufficiently
complementary to the 3' end of the HN-encoding domain to
anneal thereto under amplification conditions, a nucleotide
15 sequence encoding the human immunoglobulin hinge region γ_1
(SEQ ID NO:11), a nucleotide sequence encoding the human
CCK-8 octapeptide (SEQ ID NO:6), and a unique restriction
endonuclease cleavage site.

The PCR product (termed BoNT/A^{L-HN- γ -CCK}) is purified by
20 agarose gel electrophoresis, and cloned into a pBluescript
II SK vector. The resulting plasmid is used to transform
competent *E. coli* cells, and a preparation of the resulting
plasmid is made. The BoNT/A^{L-HN- γ -CCK} fragment is excised from
the pBluescript vector and cloned into a mammalian
25 expression vector immediately downstream of a strong
promoter. The resulting vector is used to transfect a
culture of the appropriate host cell, which is then grown to
confluence. Expression of the BoNT/A^{L-HN- γ -CCK} polypeptide is
induced, and the cells are lysed. The polypeptide is first
30 purified by gel exclusion chromatography, the fractions
containing the recombinant therapeutic agent are pooled,
then the BoNT/A^{L-HN- γ -CCK} polypeptide is further purified using

- 5 an anti-Ig affinity column wherein the antibody is directed to the γ_1 hinge region of a human immunoglobulin.

Example 2: Method of Treating a Patient Suffering from

10 Acute Pancreatitis

A therapeutically effective amount of the BoNT/A^{LHN7CCK} agent constructed and purified as set forth in Example 1 is formulated in an acceptable infusion solution. Properties
15 of pharmacologically acceptable infusion solutions, including proper electrolyte balance, are well known in the art. This solution is provided intravenously to a patient suffering from acute pancreatitis on a single day over a period of one to two hours. Additionally, the patient is
20 fed intravenously on a diet low in complex carbohydrates, complex fats and proteins.

At the beginning of treatment, the patient's pancreas shows signs of autodigestion, as measured by blood amylase levels. After the treatment regimen, autodigestion has
25 ceased, and the patient's pancreas has stabilized.

Example 3: Alternative Treatment Method

In this example, a patient suffering from acute
30 pancreatitis is treated as in Example 2, with, the therapeutic agent given continuously over a period of two weeks. After the treatment regimen, autodigestion has ceased, and the patient's pancreas has stabilized.

5 Example 4: Alternative Treatment Method

In this example, a patient suffering from acute pancreatitis is given a single pharmacologically effective amount of the therapeutic agent of Example 1 by parenteral administration.

10 Two days after the treatment regimen, autodigestion has ceased and the patient's pancreas has stabilized.

15

It will be understood that the present invention is not to be limited by the embodiments and examples described herein, and that the invention is defined solely by the claims that conclude this specification.

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